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BOTANICAL GAZETTE

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ON THE DEVELOPMENT OF CERTAIN PIPERACEAE.

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(WITH PLATES IX-X)

IN an earlier paper on *Peperomia* I stated (Johnson, 1900^a, p. 9) that *Piper* and *Heckeria* have "essentially typical Angiosperm embryo-sacs." This statement was based on observations covering the critical stages in the development of this structure. Since then I have studied the embryo-sac in these genera in detail, for the purpose of discovering any possible clue to the derivation of the peculiar type of embryo-sac found in the related genus *Peperomia*. The more important results of a study of these forms are given here, together with an account of the fate during germination of the various structures found in the fruit of *Peperomia*.

I. THE OVULE, SEED, AND FRUIT OF PIPER.

The two species of *Piper* studied were *Piper adunca* L. and a species determined as *Piper medium* Jacq. The development of the former, as far as followed, agreed closely with that of *Piper medium*; it will therefore suffice to describe and figure the latter in detail.

The flowers of *Piper medium* occur rather loosely scattered on a spike 4 to 8^{cm} in length. In the development of the flower the three outer stamens appear first, then the three inner ones. Each stamen has four pollen-sacs (*fig. 1, ps*), and the whole development of the stamens shows no noteworthy peculiarities. Soon after the stamens are formed the wall of the ovary appears

as a usually three-lobed ring just within the inner row of stamens (*fig. 2, cp*). These lobes ultimately fuse together except the stigmatic tips (*fig. 1, st*), and form a rather fleshy ovary wall, with at first a broad, somewhat capitate tip (*figs. 5, 11*). Finally the ovary becomes somewhat barrel-shaped, and when ripe is about 2^{mm} long and 1.5^{mm} in diameter, while the withered stigmas appear as slight papillae at the upper end (*fig. 14, st*).

From an early stage of development there are found in the wall of the ovary numerous scattered oil-secreting cells with nucleated protoplasts (*figs. 5, 11, oc*). In the nearly ripe fruit the carpellary tissue is differentiated into three distinct layers, the outer including the epidermis. The latter is made up of slightly elongated cells with papillose outer walls. At the base of the ovary are found a few scattered, multicellular, pointed hairs similar to those found more abundantly on the base of the spike, and to those shown for *Heckeria* in *fig. 16*. The first layer of tissue below the epidermis is of several layers of rather loose parenchyma cells, among which are scattered oil-containing cells (*figs. 11, 14, 15, cp¹*). The second tissue layer is of from five to ten layers of closely packed cells, elongated longitudinally to the fruit and without interspersed oil-cells (*figs. 11, 14, 15, cp²*). While both these outer layers of the ovary wall are continuous around the whole circumference, the third and innermost layer is discontinuous and confined to the six longitudinal grooves in the integument (*figs. 14, 15, cp³*). This layer is made up of somewhat elongated cells with trabeculate walls, thickly interspersed with oil-cells. At the border between the second and third layers and opposite the grooves in the integument occur strands of vascular tissue (*figs. 11, 14, 15, vb*), showing from three to eight or ten tracheids in transverse section. These strands continue on into the loose parenchyma of the styler region where the tracheids become considerably swollen and sometimes detached from each other (*figs. 11, 14*).

The ovule, which is terminal on the axis, arises just as the carpel lobes are closing in above. The two integuments appear nearly simultaneously just after the archesporial cell becomes distinguishable (*fig. 3, iin, oin*). The outer integument soon

becomes much longer than the inner and closes together above the latter (*figs. 5, 6*). The inner integument a little later becomes much thicker than the outer, of three layers except near the micropylar end, and finally forms the principal seed-coat (*figs. 6, 11, 12, 13, 15, in*). One or two layers of cells of the outer integument are recognizable in the ripe seed. The inner walls of the inner layer are thickened with a granular deposit resembling that found in the inner layer of carpellary cells in *Peperomia*. The primary hypodermal archesporial cell divides before the integuments have developed far, to form a tapetal cell above and the definitive archesporial cell below (*fig. 4, tp, es*). The tapetal cell later divides further to form four or five layers of cells above the embryo sac in the ripe seed (*figs. 8, 12, 13, tp*), not however, forming so sharply marked a group of cells as in *Peperomia pellucida* (Johnson, 1900^a, p. 2). The single definitive archesporial cell becomes a megaspore directly, without further division. It increases greatly in size, as does its nucleus also (*fig. 6, es*), but in so doing does not encroach upon the cells above it in the nucellus in such a way as to indicate that these are anything other than tapetal cells (*figs. 6, 7, 8, 9, es, tp*). No evidence was discovered at any stage of the formation of sister megaspores destined to final absorption by the functional one, as in so many Angiosperms.

The division of the megaspore occurs soon after the inner integument has closed in to form a narrow micropyle above the nucellus. This division occurs in the manner so frequently described. Of the first two nuclei formed one goes to each pole and there each divides (*fig. 7*). The four nuclei thus formed are frequently found grouped rather closely in the middle of the embryo sac (*fig. 8*), or in other cases we find a pair near each pole. The eight nuclei formed at the next division soon group themselves in the typical manner to form the ripe embryo sac (*fig. 9*). The exact derivation of the polar nuclei has not been made out with absolute certainty, but there seems no reason to doubt from the condition shown in *fig. 9* that it is as commonly found in the Angiosperms.

In the ripe embryo sac the large egg and the two smaller per-

sistent synergids have well-marked cell walls (*fig. 10*). The large endosperm nucleus is found in a considerable mass of protoplasm, usually near the middle of the embryo sac (*fig. 10, espn*). The distinct antipodals early become sunken in the very bottom of the embryo sac. They seem never to increase in number nor greatly in size, but persist, though slightly crushed, even in the ripe seed (*figs. 12, 13, 14*). During the ripening of the embryo sac it continues to broaden, and when the endosperm begins to form it is nearly globular (*fig. 13*), and in the ripe seed it may be even broader than long (*fig. 14*).

No clear evidence of fertilization or even of the penetration of the pollen tube to the embryo sac has been obtained. Many pollen grains were found on the stigma, some of which had evidently begun to germinate.

In the further development of the embryo sac the endosperm nucleus is at first most active. This nucleus divides repeatedly by mitosis. The daughter nuclei become distributed throughout the peripheral cytoplasmic layer of the embryo sac (*fig. 12*), and after twenty or more free nuclei have been formed cell-walls appear between and about these, and a peripheral layer of cellular endosperm is formed (*fig. 13*). Later the central vacuole is filled up with endosperm cells (*fig. 14*), probably by the division of those of the peripheral layer, since no free nuclei were discovered in the vacuole within the first layer of endosperm cells formed. In the ripe seed the endosperm forms a somewhat top-shaped mass of several hundreds of cells (*fig. 14*).

During this development of the endosperm the egg changes but little except in size at first (*figs. 10, 12, 13*), but later it divides up to form a globular embryo, which in the oldest seeds seen (probably ripe ones) consisted in longitudinal section of thirty or forty cells, and showed no differentiation save a slightly developed suspensor (*fig. 14*).

The synergids, like the antipodals, are long persistent (*figs. 12, 13*), though the former were not seen in the ripe seed.

In the mature seed the embryo is about 0.06 mm in diameter, the endosperm is 0.3 mm , and the whole seed from micropyle to basal wall 1.7 mm long by 1.2 mm broad in the middle.

The contents of the cells of the embryo in the seed seem to be chiefly protoplasmic. The cells of the endosperm possess large nuclei and are filled around this with a substance evidently chiefly proteid in nature, and probably similar in function to that to be mentioned in *Heckeria* and *Peperomia*. From the time the eight-nucleate embryo sac is formed the nucellus swells rapidly, and by the time the endosperm-nucleus begins to divide the cells of the nucellus are beginning to fill up with starch. In the ripe seed the cells of the nucellus are solidly packed with starch (*figs. 14, 15, psp*), except the cells near the micropyle and those of the peripheral layer of the nucellus generally, in which the starch grains are smaller and there is a somewhat larger proportion of protoplasmic contents. The crushed and distorted nuclei of the starch-filled perisperm cells are distinguishable even in the mature seed.

No seeds of this species capable of germination were available, but it is natural to suppose that the process of sprouting must resemble closely that to be described in *Heckeria* and *Peperomia*, in which genera the structure of the ripe seed is essentially that just described.

II. THE DEVELOPMENT AND GERMINATION OF THE SEED OF HECKERIA.

Material of both *H. umbellata* and *H. peltata* was available, but a complete study was made of the former only.

The ovary of this form arises as a ring-like outgrowth of the axis of the spike above a slightly peltate bract (*fig. 16*), and between the two stamens, which appear somewhat earlier than the carpels. The edges of this ring soon become three-lobed, probably indicating the presence of three carpels in the ovary; and as the walls of the ovary close in above, each lobe stretches out to form a somewhat elongated division of the stigma (*figs. 22, 24, st*). From the time the carpels close in above the ovule until the fruit is ripe, there are found scattered through the carpellary tissue oil-secreting cells, the protoplasts and nuclei of which remain active until a very late stage of development (*figs. 17, 22, 24, oc*). In the mature fruit these are still present and

completely filled with globules of an oily or waxy substance. The stigmas of the ripe fruit are much shrunk (*fig. 29*). The walls of the ovary near the base of the ripe fruit are of six or eight cells in thickness, the cells being generally elongated with the axis of the fruit but not clearly differentiated into layers as in *Piper medium*. The inner cell-wall of the inner layer becomes irregularly thickened with granular material, as in the case of *Peperomia pellucida*. About the upper end of the seed the tissue of the carpels becomes thickened to a mass of rather loosely packed, thin-walled parenchyma cells with oil-cells scattered among them. There are no definite vascular bundles above the very base of the fruit, but scattered tracheids occur, especially in the region just below the stigma. The outer cell-walls of the epidermal layer of the ovary are finely granulated. The hairs which occur abundantly on the bracts (*fig. 16*) are wanting on the ovary. The nucellus of the ovule arises in a strictly basal position, at about the time the walls of the ovary are closing together above. The single hypodermal archesporial cell is distinguishable somewhat later, and is undivided up to the time when the integuments appear (*fig. 17, arsp*). A tapetal cell and the definitive archesporial cell are formed in the ordinary manner (*fig. 18*), and before the integuments have closed above the nucellus the tapetal cell divides to two or three layers (*fig. 19, tp*), which persist in the ripe seed (*figs. 23, 27, 29*).

The two integuments arise in the usual way, and nearly simultaneously, from the base of the nucellus (*figs. 17, 18*). The outer integument develops far enough to close in above the inner (*figs. 19, 22*), and becomes two or three cells thick, but only the inner walls of the inner layer ever thicken greatly, and in the mature seed only the inner layer of cells of this integument is distinguishable, the outer layer being crushed against the surrounding carpellary tissue. The inner integument closes to a narrow micropyle, the cells of which finally grow over to interlock closely from opposite sides (*fig. 27*). This integument soon becomes three-layered, or it may become six-layered above, and in the ripe seed the walls of all these layers of cells become thickened and brown to form the principal seed coat.

The inner layer is made up of radially elongated, prismatic cells, the middle layer of periclinally expanded ones, and the outer of more or less cubical cells (*figs. 24, 25, 27*).

The mature seed is distinctly triangular in transverse section, but the surface of the fruit may be five or six-angled from the pressure of adjacent fruits upon each other in the closely packed spike.

The definitive archesporial cell gives rise immediately to a single megaspore, no indication of degenerating sister megaspores being seen (*figs. 19, 20, 21*). This single megaspore, after enlarging slightly, gives rise to two, four, and finally eight nuclei in the typical manner (*figs. 20, 21, 23*). The large egg and the two synergids, all with distinct cell-walls, occupy the upper end of the elongated embryo sac. The polar nuclei fuse to form a large endosperm-nucleus (*figs. 23, 25*), which may be located near either the base, middle, or upper end of the embryo sac. The antipodal nuclei collect at the extreme base of the sac and do not develop cell walls until somewhat later than the egg and synergids (*figs. 23, 25*).

At about the time of fusion of the polar nuclei the embryo sac ceases to elongate and begins to broaden rapidly, so that the mature embryo sac is nearly spherical in form (*fig. 25*). At this stage the egg and synergids are somewhat flattened against the upper end of the embryo sac, and the very large antipodals bulge into the cavity of the sac from below (*figs. 24, 25*).

As in the case of *Piper medium* mentioned above, the synergids and antipodals are long persistent (*figs. 26, 27*), though apparently not quite so long as in the latter genus. The number of antipodals found below the endosperm mass in the nearly ripe seed is frequently but three; sometimes, however, there may be as many as six or eight (*fig. 28*), which probably arise by the division of the three primary ones, after their cell walls are formed. The prominence of the antipodals here suggests that their function is an important one. They are placed at the base of the embryo sac, in what is probably the direct line of food transit to the sac, and perhaps play a part in the transmission or possibly in the elaboration also of this food material, though the

shape of the older embryo sac seems to indicate that their power of breaking down and absorbing surrounding tissues is not as great as in many other known cases.

The embryo sac becomes filled with cellular endosperm before the egg divides (*fig. 26*). The very first division of the endosperm nucleus has not been seen, but when four nuclei have been formed these are found to be separated by cell walls, so that I believe that a cell wall is formed immediately after the first and each succeeding division of the endosperm nucleus, and thus the endosperm is cellular from the very first. This feature of the development at once recalls the case of *Peperomia pellucida* (Johnson, 1900^a), but differs strikingly from that of the more closely related genus *Piper*, as described above. Similar marked differences in the mode of formation of the endosperm in closely related genera have been already noted by Hofmeister (1859, p. 555 *et seq.*), and Hegelmaier (1885, p. 92). Evidently peculiarities of this sort cannot be taken as adequate indications of relationship, as has been recently suggested by Cook (1902) in the case of *Nymphaea* and *Sagittaria*.

The cells of the endosperm of *Heckeria* are at first largely vacuolated and possess large nuclei. The first cell-walls formed radiate from the egg (*fig. 26*), but later the walls come in somewhat irregularly (*figs. 27, 28*). In the ripe seed there are two hundred or more endosperm cells surrounding the embryo, in from two to five layers, and occupying a broadly top-shaped space at the upper end of the seed (*fig. 29*). The vacuoles of the endosperm cells at this time are filled closely with proteid granules, except some of the cells near the embryo, which have but little contents.

The first division of the egg occurs some time after the formation of endosperm begins, whether in consequence of fertilization or not has not been made out with certainty. The direction of the first wall in the few cases seen is oblique, and the arrangement of the cells in the many older embryos seen make it certain that this is the general rule (*figs. 27, 28*.) In the ripe seed the embryo consists of several hundreds of small elongated cells, making a globular mass about 0.05^{mm} in diameter, undifferen-

tiated except for a slight projection above, which perhaps is a secondarily developed, rudimentary suspensor (*fig. 29*). As the embryo pushes down into the endosperm the latter seems to close in above so that the embryo often comes to lie nearly centrally in the endosperm.

The germination of the seed in *Heckeria umbellata* (*figs. 30, 31*) is essentially like that of *Peperomia pellucida*, and will be mentioned in connection with the discussion of this process in the latter genus.

III. THE GERMINATION OF THE SEEDS OF PEPEROMIA AND HECKERIA.

The ripe fruit of *Peperomia pellucida* is oval in form, with a slightly pointed upper end, being about 0.85^{mm} long and 0.6^{mm} wide. It is made up, beside the seed, of four or five layers of carpellary tissue. The outer or epidermal layer is made up of bulging, thin-walled cells interspersed with knob-like glandular hairs, perhaps hydathodes in function (*fig. 32*.) The inner layer of the ovary-wall is of very large cells, with coarsely reticulate outer walls and with the basal walls irregularly thickened with granular material (*figs. 32, 33*). Between this layer and the epidermis are two or three layers of delicate, flattened cells.

The seed proper is 0.6^{mm} long and 0.45^{mm} in transverse diameter. It is surrounded by a single integument, the two cell-layers of which have their walls thickened and of a very dark color. The inner walls of the outer layer of the integument fit against the cells of the perisperm, especially at the upper end of the seed, with a very wavy outline (*figs. 32, 33*). In an earlier paper (Johnson, 1900^a, *fig. 15*) I was misled, by the much more regular line of cell-walls separating the outer layer of perisperm cells from those within, into thinking this the line of separation between integument and perisperm. The interpretation given above is however the correct one, as has been shown by Campbell (1901, p. 3), and the lower index line from *int* in the *figure 15* referred to above should run only to the inner, wavy border of the inner of the two darkly shaded cell-layers. All of the cells represented as containing starch in this figure belong to the perisperm. The great bulk of the seed within the integuments is

made up of nucellar tissue, or perisperm, richly stored with starch (*fig. 32*), the grains of starch in the outer cells being finer and less abundant than in the inner ones.

Near the upper end of this perisperm mass, separated from the integument by but two or three layers of cells, lies the somewhat oblately spheroidal mass of endosperm (*fig. 32*). This mass is 0.09^{mm} to 0.1^{mm} in diameter, and is made up of forty or more cubical cells with darkly staining contents, including abundant aleurone grains (Johnson, 1900^a, *fig. 13*).

The embryo is rounded or slightly elongated, without a well-marked suspensor or other sign of differentiation, and lies at the upper border of the endosperm (*fig. 32*). It is surrounded laterally and below by one or two layers of endosperm. It is 0.035^{mm} to 0.04^{mm} long, and is made up of about fifteen cells.

In many sections of the ripe seed a group of cells, corresponding in position to a synergid, is found near the upper end of the embryo, and seems entirely distinct from the endosperm (Johnson, 1900^a, p. 6). Observations made thus far fail to show anything peculiar in the fate of this group of cells during germination. These cells and the six other peripheral cells outside the endosperm (Johnson, 1900^a, *fig. 13*) are soon crushed aside by the swelling of the latter in sprouting.

Seeds of *Peperomia pellucida* collected in Jamaica in August 1900 germinated readily in October of that year. They therefore do not need this long rest apparently necessary for the seeds of *Saururus cernuus* (Johnson, 1900^b, p. 369). The exact course of development, that is the time of ripening and the length of rest required by the seed under the normal conditions, must of course be followed out in the tropics. In Baltimore the behavior of the seeds is as described below, when allowed to germinate on wet filter paper or chopped Sphagnum, at a temperature of 15° to 20°, before an unshaded window, but not in direct sunlight.

No external change whatever is visible in the seed for about a week, except an immediate slight swelling of the carpellary tissue, but important changes are going on within. The embryo grows to 0.1^{mm} in diameter, remaining globular in shape, and is

at this time made up of two hundred and fifty cells or more (*fig. 33*). The endosperm cells divide actively, chiefly by anticlines, swelling meanwhile to encroach upon the perisperm, and upon reaching a diameter of 0.3^{mm} begin to burst the integument near the micropylar end, but remain as a complete sac about the embryo (*fig. 34*).

As germination continues the embryo elongates, the two cotyledons form below, and the primary vascular strand of the hypocotyl appears (*fig. 35*). The endosperm uses up the contents of the surrounding perisperm cells, crushing in their walls at first, and then pushes out above between the rent edges of the integument and carpel (*fig. 35*). In so doing it either pushes aside or carries up with it the persistent group of tapetal cells and the overlying stigmatic regions of the carpel. The embryo remains enclosed within the endosperm sac until it is 0.5^{mm} long or more, at which time the cotyledons are 0.15^{mm} or 0.2^{mm} long, and have each a rudimentary vascular bundle connecting with that of the hypocotyl.

Soon after this size is reached the rapid elongation of the embryo pushes at first the tip and then the whole of the radicle through the upper part of the sac of endosperm (*fig. 36*). Still later by the elongation of the cotyledons all but the tips of the latter are pushed out of the endosperm (*fig. 37*). A swelling of the radicle, somewhat below the middle, first indicates the limits of root and hypocotyl. Below this root hairs soon appear abundantly, and later secondary lateral roots (*figs. 37, 39*). Above on the hypocotyl and cotyledons, even while the latter are still enclosed by the endosperm, numerous glandular hairs and many stomata are formed (*figs. 37, 38*). Within the cotyledons between the vascular bundle and the upper surface of the leaf there is found at this stage a single layer of cells with darkly staining contents whose exact nature and function are as yet undetermined (*fig. 38*).

As the root of the seedling elongates it curves downward to enter and anchor itself in the substratum by numerous root hairs and secondary roots. Then the hypocotyl elongates and thus raises the cotyledons with the adhering endosperm in the air

(fig. 37). The endosperm at this time is closely in contact with the tips of the cotyledons and even forces its way in between them somewhat (figs. 35, 36). At the outer or upper part of the endosperm it is somewhat thickened and forms thus a collar which tightly grasps the tips of the cotyledons and fills completely the rent in the seed-coat (figs. 37, 38, esp). This collar evidently serves not only to hold the seed on the tips of the cotyledons, but also to prevent the exit and loss of any dissolved food material on its passage between the perisperm and the embryo. This collar must also prevent pretty effectually the entrance of water from without to dilute this food material, or of bacteria and fungi to destroy it. The walls of the endosperm cells are comparatively thin throughout, and no indication of thinner spots in their walls or those of the cells of the cotyledons was seen.

The endosperm cap, pushed on by the swelling cotyledons, finally crushes in the previously emptied perisperm cells, until it fills about half the bulk of the seed. After this the absorption may go on until practically all the starch is removed from the remaining perisperm cells, while the walls of these cells remain distended just as when full of food material (figs. 36, 38). When the seedling is about 15^{mm} high, the starch of the perisperm being then nearly exhausted, the wasted remains of the seed and fruit slip off from the tips of the cotyledons. Then the curve in the hypocotyl straightens out, and the cotyledons open out widely to allow the still very small plumule to develop and expand (fig. 39). During the transference of the starch from the perisperm to the embryo, cells of the former can be seen in which the end near the endosperm is nearly devoid of contents, except for a faintly staining, apparently slimy substance, while the opposite end is still closely packed with starch grains. The chemical changes taking place during this process and the exact mode of transmission have not been made out satisfactorily as yet, but it is hoped that these may be followed out in detail as soon as a supply of fresh seeds can be obtained.

The ripe seed of *Heckeria umbellata* differs from that of *Peperomia pellucida* somewhat in shape and size (figs. 29, 32), but

chiefly, for our present purpose, in the considerably more massive embryo of the former. This is however still undifferentiated except for the slightly developed suspensor (*fig. 29*). The process of germination is also practically identical in both species, except that the embryo becomes slightly more differentiated in *Heckeria* before bursting through the endosperm (*figs. 30, 31, 34, 37, 38*).

The point of chief interest in the germination of these genera, in addition to the deferring of the formation of the organs of the embryo to the time of germination, is the behavior of the scanty endosperm. The endosperm of *Peperomia* and *Heckeria* does not at any time during its development contain any considerable amount of starch, though a few scattered grains may appear in it during germination. These latter are perhaps portions of the carbohydrate which are temporarily fixed in solid form during transmission through the endosperm. The cells of this tissue in the ripe seed are pretty rich in protoplasmic contents, with large nuclei (Johnson, 1900^a, *fig. 13*), and the vacuoles are filled closely with aleurone grains. These facts suggest that the chief function of the endosperm in these genera is not the storage of ordinary reserve food material, as this function is served in these seeds by the perisperm. The later history of the endosperm here indicates, though absolute proof must come from careful chemical work, that it really serves as a digestive apparatus for breaking down and absorbing the material stored in the surrounding perisperm and passing it on to the embryo. If this interpretation is the correct one, then the endosperm here fulfills the function which in many other seeds is performed by a part of the embryo itself, as for example the scutellum of the grasses (Haberlandt, 1896, p. 212). The aleurone and denser protoplasmic contents of the endosperm cells gradually disappear as germination proceeds, but the cell-walls remain plump and uncrushed till a comparatively late period of development, except a few cells of the inner layer near the tips of the cotyledons (*figs. 36, 38*). The fact that the cell-contents disappear first from the inner layer of the endosperm might suggest that this material goes directly to the embryo, but it seems quite

as possible that it may pass to the outer layer and thus keep this in condition to continue the absorption of starch from the perisperm. Only a careful chemical study of the cell-contents of all these tissues during the progress of germination can decide these questions definitely.

The restriction of the functions of the endosperm to the passing on, during germination, of the food material without to the embryo within has, so far as I have been able to learn, been pointed out in *Saururus cernuus* only (Johnson, 1900^b, p. 370). Humphrey (1896, p. 16) suggested that the single, aleurone-containing layer of endosperm in *Canna* might have some such function, but did not follow this out in germination. In the germinating seeds of *Canna* I find that this layer of endosperm persists till a late stage of this process as a sac about the haustorium, the relation being such that material from the perisperm must pass through the endosperm to get to the embryo, and it seems probable that it may play the same part as that suggested in *Peperomia* and *Heckeria*.

In many other families it has been shown, by Hartz (1885) and others, that the storage tissue in the seed outside the embryo is perisperm, and not endosperm, as stated in the older (and many of the newer) books. This is true, for example, of the Polygonaceae, Chenopodiaceae, Phytolaccaceae, Caryophyllaceae, and others, and, contrary to the accepted authorities, there is present in a considerable number of these forms that I have studied a small amount of endosperm also. In all cases of these perisperm-containing seeds which were sprouted, the endosperm was found to persist for some time during germination, and thus to be capable of taking part in the transference of food material to the embryo.

Observations thus far made lead me to believe that in the perisperm-containing seeds mentioned the embryo sporophyte of the second generation is never nourished by the parent sporophyte directly, but always through the intermediate gametophyte. In general, then, we find that the food substance supplied to the embryo by the nucellus may pass through the endosperm and be stored in the embryo during the ripening of the seed, as in

Cucurbita and Phaseolus ; or secondly, the food may be stopped in transit between the nucellus and the embryo, and stored in the endosperm, there to be held during the resting period of the seed, and delivered over to the embryo only at the time of sprouting, as in Ricinus, Zea, and apparently all Gymnosperms; or finally, the food supply for the developing embryo may be stored in the nucellus itself until the time of germination, when it is passed on to the embryo through the endosperm, as in Saururus, Peperomia, Phytolacca, Canna, and others.

IV. SUMMARY AND CONCLUSIONS.

Campbell has shown (1899, p. 453) that the nearly ripe embryo sac of *Peperomia pellucida* differs from that of all other Angiosperms studied up to that time in the presence of sixteen free nuclei derived from the megaspore nucleus. The writer found the same species to be peculiar also in the formation of the endosperm nucleus by the fusion of eight of these embryo sac nuclei, and in the persistence of seven others of these near the wall of the sac, one of them near the egg in the position of a synergid (Johnson, 1900^a, p. 4). The ripe seed has a single integument, contains an undifferentiated embryo of about fifteen cells surrounded by a very small endosperm, which is cellular from the two-nucleate stage on, contains chiefly aleurone, and is surrounded in turn by an abundant perisperm stored with starch.

The study of several other species of *Peperomia* by Campbell and the writer has shown that these agree with *P. pellucida* in the features noted.

The development of the ovary, ovule, and embryo sac in *Piper* and *Heckeria* differ widely in several respects from that found in the related genus *Peperomia*. The ovary in both genera seems to be syncarpous. Two integuments are found in both. A tapetal cell and a single megaspore are formed from the archesporial cell in each genus. The megaspore gives rise in the usual way to a seven-nucleate embryo sac. The antipodals and synergids are long-persistent. The embryo in the ripe seed is very small, of forty or more cells in cross section, and globular except for a very short suspensor.

The endosperm-nucleus of *Piper* divides to twenty or more free nuclei, and then simultaneously cell-walls are formed about all of these. In *Heckeria* the endosperm-nuclei are separated from the beginning by cell-walls. Both genera resemble *Peperomia* in that the endosperm is comparatively slight in amount in the ripe seed and contains no starch, an abundant supply of the latter being stored in the surrounding perisperm.

In the germination of *Peperomia* and *Heckeria* the swelling of the endosperm and embryo bursts the seed-coats, and the endosperm protrudes through the rent as a sac which continues to surround the embryo until after root and cotyledons are differentiated. The embryo is truly dicotyledonous. The root finally pushes out through the endosperm, but the latter remains about the tips of the cotyledons and imbedded in the seed till all the starch of the perisperm is absorbed.

The present studies have failed to show, in close relatives of *Peperomia*, any peculiarities in the development of the embryo sac which are clearly intermediate between that found in this genus and that of typical Angiosperms.

The striking differences in the mode of formation of the endosperm in these three related genera shows again, as has been demonstrated by Hofmeister (1859) and Hegelmaier (1885), that characters of this kind are often of no value as indications of relationship.

The position taken by the writer in an earlier paper (Johnson, 1900^a, p. 9), that these peculiarities in *Peperomia* are secondary, a view supported by Strasburger (1900, p. 293) and Goebel (1901, p. 806), seems still the most reasonable one.

The case of *Gunnera*, where Schnegg (1902) has shown that the embryo sac contains sixteen or more nuclei, and that the endosperm nucleus is formed by the fusion of eight or ten of these, might at first sight seem to indicate that we have here again the persistence of a character which Campbell believes to be primitive. A consideration of the evidently distant relationship of these genera, however, together with the fact that no other Angiosperm known shows any relic of this supposed primitive type, makes it probable that *Peperomia* and *Gunnera* have secondarily and independently developed this type.

As to the systematic position of *Peperomia*, the writer sees at present no reason to doubt that it is properly placed among the Piperaceae. Whether the Piperales as an order are to be regarded as very primitive forms is not so clear. It is, of course, possible that the absence of floral envelopes here is primitive. The presence of perisperm in the seed is a character found in many of the simpler orders of Angiosperms, but it has probably been secondarily acquired, since we find nothing in the higher Pteridophytes or Gymnosperms to suggest that it is primitive. A survey of the Dicotyledons shows that perisperm is at present known in the seeds of Piperales, Aristolochiales, Polygonales, Centrospermae, and Ranales. All of these orders, as has been pointed out by Schimper and Lesquereux, are old geologically, and possibly represent branches of a single stock or phylum of the Dicotyledons (see Bessey, 1897, p. 33). I am inclined to believe, as many of the older systematic writers have held, that the Piperales are much more closely related to the Polygonales than the position assigned them by Engler (1898) indicates.

Within the Piperales it is evident that the flowers of *Piper* and *Heckeria* are more complex in structure (probably primitively so) than those of *Peperomia*. This is indicated by the syncarpous ovary and the presence of two integuments. It is not clear to the writer that *Saururus* is "distinctly more specialized" than some of these higher Piperaceae, as suggested by Campbell (1901, p. 114), nor that the Saururaceae are derived from the Piperaceae.

The morphological features of the germination of the seed in *Peperomia* and *Heckeria* indicate that the aleurone-containing endosperm of these forms acts as a digesting and absorbing apparatus for transferring the starch stored in the perisperm to the embryo.

In several other genera, of the Cannaceae, Polygonaceae, Phytolaccaceae, Caryophyllaceae, and others, a thin layer of endosperm separates perisperm and embryo and seems to serve the function above mentioned. The embryo sporophyte is perhaps everywhere nourished through the gametophyte and not by the parent sporophyte directly.

I am under obligation to Captain J. Donnell Smith for the freest use of his library and herbarium material, and to Mr. W. C. Coker for collecting seeds of *Peperomia* and *Heckeria* in Jamaica.

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EXPLANATION OF PLATES IX-X.

Abbreviations used: *ant*, antipodal; *arsp*, primary archesporial cell; *br*, subtending bract; *cot*, cotyledon; *cp*, carpel; *cp*¹, external layer of carpellary

tissue (including epidermis); *cp*², middle layer of carpellary tissue; *cp*³, inner layer of the same; *em*, embryo; *es*, embryo sac; *esp*, endosperm; *esp**pn*, endosperm nucleus; *hy*, hydathode; *iin*, inner integument; *in*, integument; *mp*, micropyle; *nc*, nucellus; *o*, ovum or egg; *oc*, oil-containing cell; *oin*, outer integument; *osp*, oospore; *plu*, plumule; *pn*, polar nucleus; *ps*, pollen sac; *psp*, perisperm; *rh*, roothair; *sg*, synergid; *st*, stigma; *sta*, stamen; *sto*, stoma; *tp*, tapetal cells; *vb*, vascular bundle tissue.

All figures are camera drawings, and all are from microtome sections except *figs.* 1, 37, and 39.

Figs. 1-15. Piper medium Jacq.

FIG. 1. Superior view of young flower; the stigmas are often three in number. $\times 30$.

FIG. 2. Tranverse section of an ovary slightly younger than that shown in *fig.* 5, showing indications of the three constituent carpels. $\times 50$.

FIG. 3. Longitudinal section of ovule at the beginning of formation of integuments. $\times 360$.

FIG. 4. Similar section after the formation of tapetum. $\times 360$.

FIG. 5. Longitudinal section of ovary when integuments have just closed in at the micropyle. $\times 50$.

FIG. 6. Longitudinal section of ovule from such an ovary. $\times 360$.

FIG. 7. Longitudinal section of a nucellus containing a four-nucleate embryo sac. $\times 360$.

FIG. 8. Similar section at a slightly later stage. $\times 360$.

FIG. 9. A similar section of an eight-nucleate embryo sac. $\times 360$.

FIG. 10. Longitudinal section of a ripe embryo sac; the nuclei of the synergids from the adjoining section shown in dotted lines. $\times 360$.

FIG. 11. Longitudinal section of a nearly full grown fruit. $\times 30$.

FIG. 12. Detail of micropylar region of same, with embryo sac showing multinucleate non-cellular endosperm. $\times 360$.

FIG. 13. Longitudinal section of somewhat older embryo sac, and adjoining portions of nucellus, showing peripheral layer of cellular endosperm; the synergids indicated in dotted lines are from the adjoining section. $\times 175$.

FIG. 14. Longitudinal section of ripe food and seed, showing relative development of embryo, endosperm, and perisperm. $\times 30$.

FIG. 15. Transverse section at about the middle of ripe fruit. $\times 30$.

Figs. 16-31. Heckeria umbellata L.

FIG. 16. Part of longitudinal section of spike, passing through three ovaries and their subtending bracts. $\times 360$.

FIG. 17. Longitudinal section of an older ovary and the enclosed ovule. $\times 175$.

FIG. 18. Longitudinal section of still older ovule showing tapetum. $\times 360$.

FIG. 19. Longitudinal section of still older ovule. $\times 360$.

FIG. 20. Longitudinal section of ovule with four-nucleate embryo sac. $\times 360$.

FIG. 21. Longitudinal section of slightly older ovule. $\times 360$.

FIG. 22. Longitudinal section of fruit with nearly ripe embryo sac. $\times 125$.

FIG. 23. Detail of embryo sac from section shown in last figure. $\times 360$.

FIG. 24. Longitudinal section of fruit with ripe embryo sac. $\times 50$.

FIG. 25. Embryo sac of same, with surrounding portions of nucellus and inner integument; synergids from adjoining section. $\times 360$.

FIG. 26. Longitudinal section of an older embryo sac with a several-celled endosperm. $\times 360$.

FIG. 27. Similar section of still older embryo sac with twelve-celled embryo. $\times 360$.

FIG. 28. Similar section showing six antipodals. $\times 360$.

FIG. 29. Longitudinal section of ripe fruit. $\times 55$.

FIG. 30. Longitudinal section of germinating seed; the perisperm above the dotted line has been exhausted of starch. $\times 75$.

FIG. 31. Similar section at a later stage of germination. $\times 30$.

Figs. 32-39. Peperomia pellucida Kunth.

FIG. 32. Longitudinal section of ripe fruit. $\times 55$.

FIG. 33. Terminal part of a similar section at an early stage of germination. $\times 175$.

FIG. 34. Longitudinal section of same at still later stage, showing tip of integument and carpel burst off by swelling of endosperm and embryo. $\times 30$.

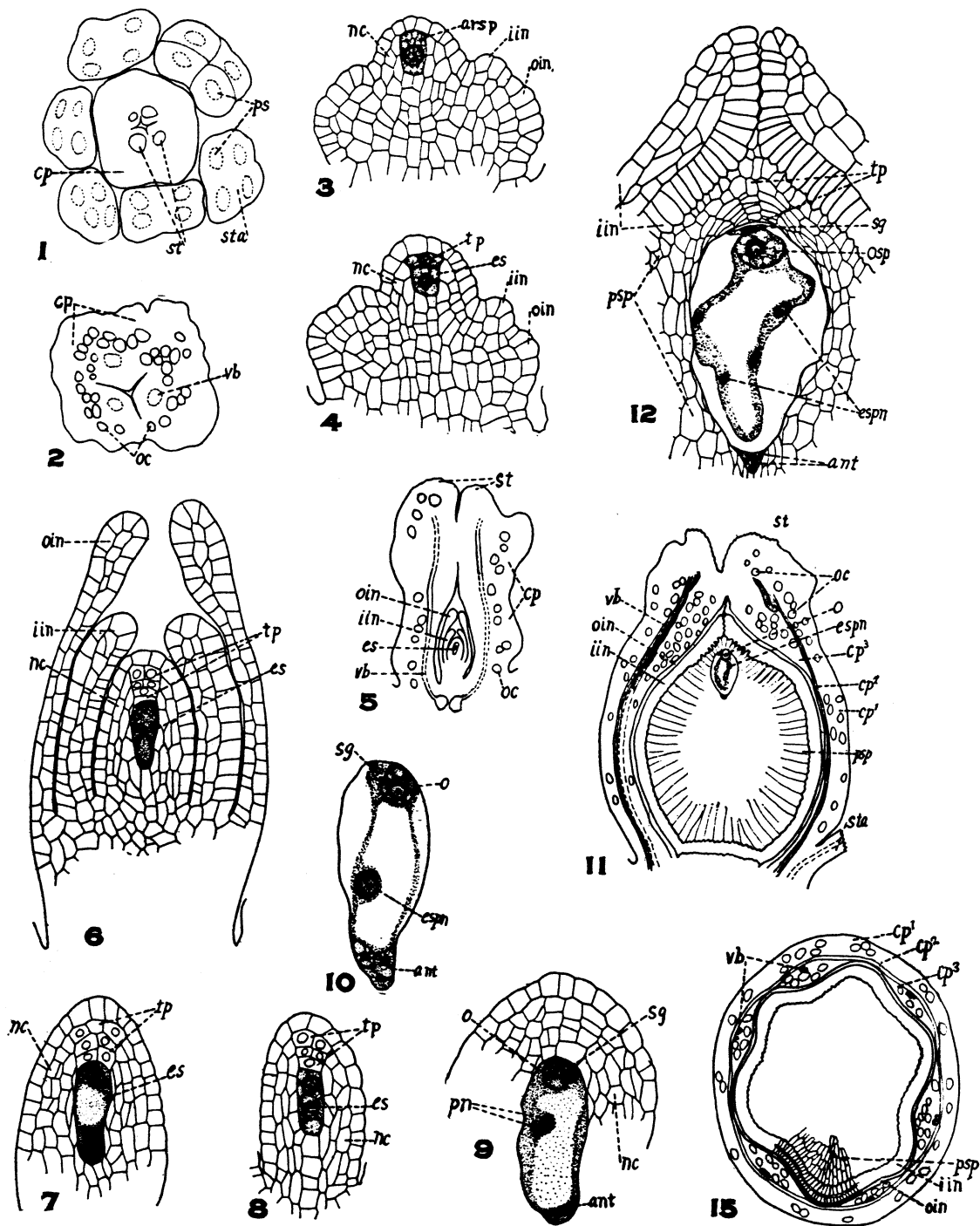
FIG. 35. Detail of embryo and endosperm of same. $\times 175$.

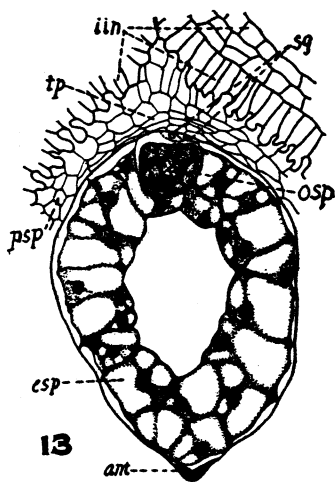
FIG. 36. Longitudinal section of germinating seed (with adhering carpelary tissue), showing radicle pushed out through endosperm. $\times 55$.

FIG. 37. Surface view of seedling with seed about ready to drop from the cotyledons. $\times 30$.

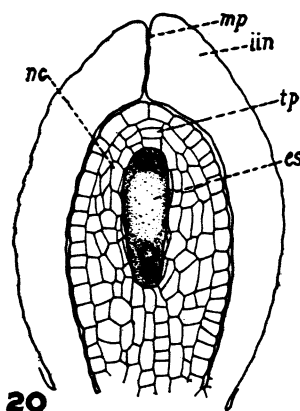
FIG. 38. Detail of same, showing seed and tips of cotyledons. $\times 50$.

FIG. 39. Seedling with expanded cotyledons, showing plumule. $\times 5$.

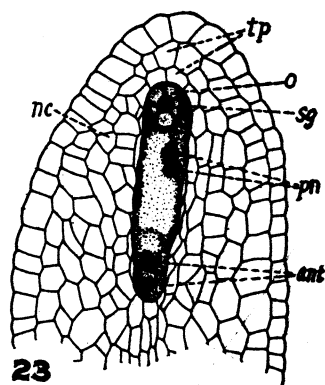




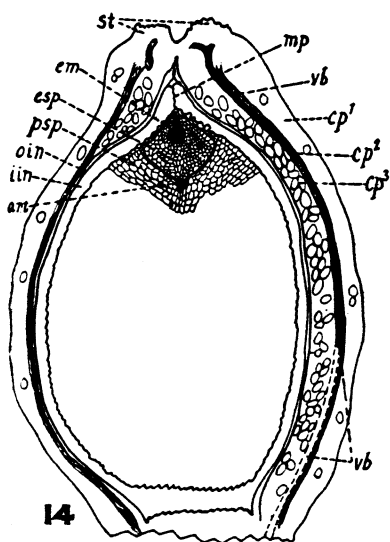
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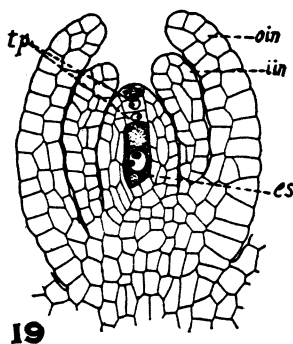
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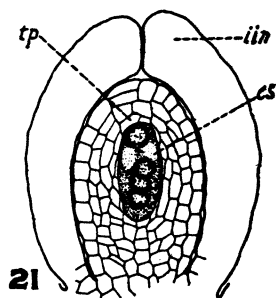
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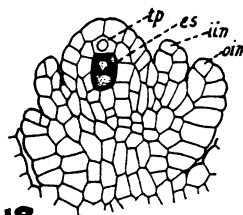
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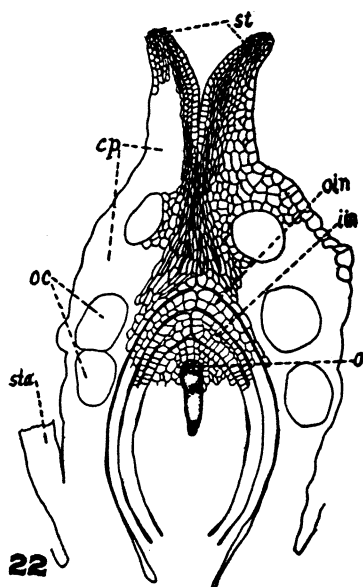
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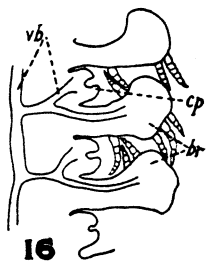
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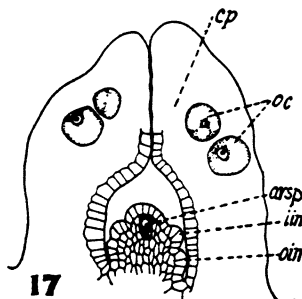
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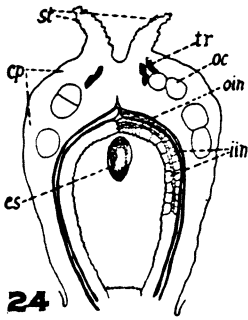
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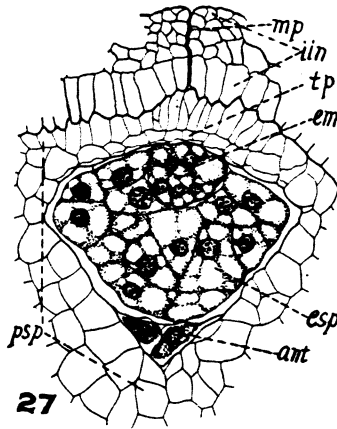
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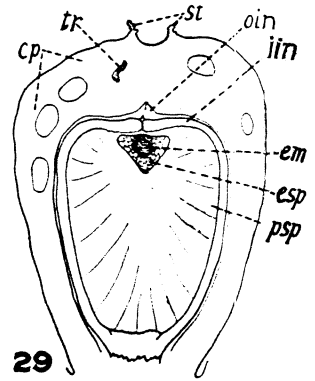
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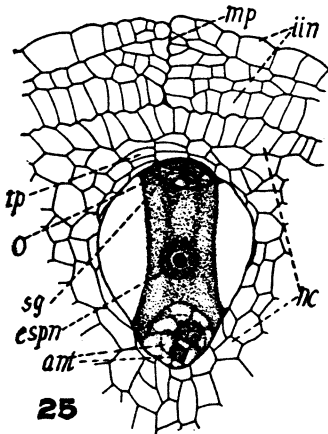
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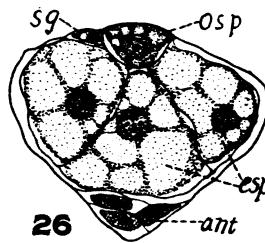
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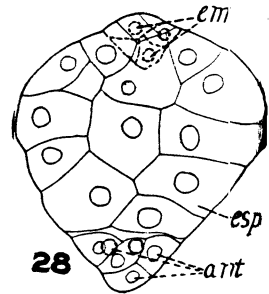
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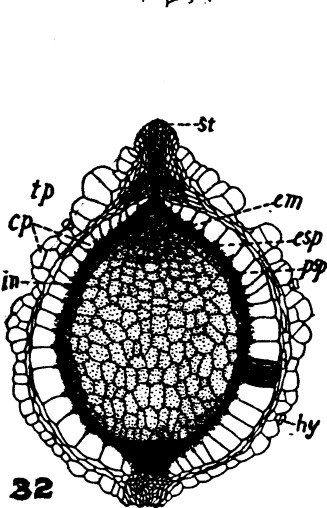
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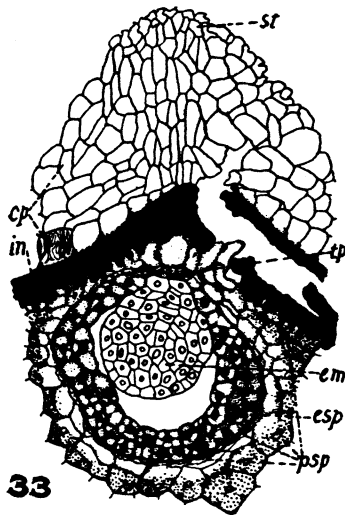
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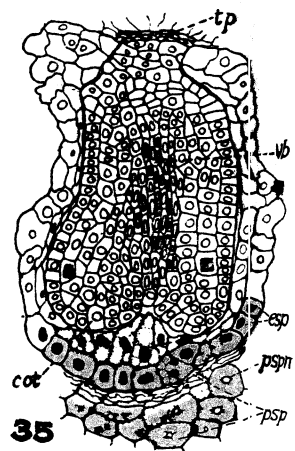
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